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REVIEW

Passage to the Terrestrial Life in Amphibians
II. Endocrine Determinism

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ABSTRACT—In many amphibians, the departure from water associated with the metamorphic events is controlled by a number of hormones. The most important are the thyroid hormones. After they bind to the nuclear receptors, the thyroid hormones trigger a number of mechanisms by acting at the genome level. These hormones control the synthesis of many specialized proteins during cell differentiation and degeneration. Thyroid hormone activity is a necessary condition for metamorphosis and departure from water in many amphibians. However, it is not in itself sufficient and other hormones intervene also. Cortical steroids such as corticosterone or aldosterone play a part in up-regulating metamorphosis. On the contrary, prolactin and growth hormone can inhibit certain metamorphic events in larvae, thus maintaining them in an aquatic environment. Moreover, neurohypophyseal hormones, aldosterone or prolactin regulate the hydromineral equilibrium which optimizes water conservation in the metamorphosed animals, while they migrate towards the terrestrial habitat. Finally, a complex endocrine determinism presides over the environmental transitions observed in modern amphibian development.

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INTRODUCTION

Departure from water in most amphibians is prepared by a number of transformations that modify the larvae's organization during metamorphosis.

Since Gudernatsch's studies [48], it is admitted that amphibian metamorphosis is triggered by an increase in the concentration of circulating thyroid hormones (3,5,3'-triiodothyronine or T_3, 3,5,3',5'-tetraiodothyronine or thyroxine or T_4), due to an increase in the activity of the thyroid gland (Fig. 1). Thyroidectomy or the administration of antithyroid agents (thiourea) eliminates metamorphosis in the larvae, which nevertheless continue to grow. On the contrary, treating the larvae with thyroid hormones anticipates the phenomenon in anurans as well as in urodeles. The triggering of metamorphosis by thyroid hormones in amphibians is the oldest known endocrine effect in poikilothermic vertebrates.

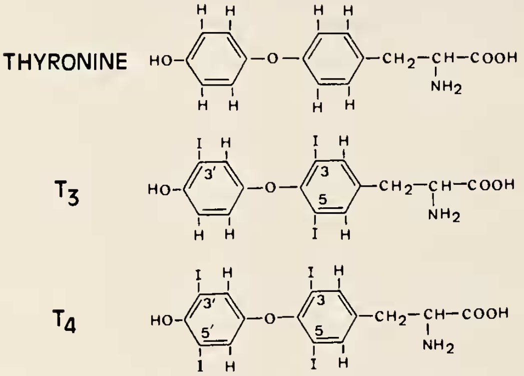


Fig. 1. The two thyroid hormones (3,5,3'-triiodothyronine or T_3, and 3,5,3'5'-tetraiodothyronine or thyroxine or T_4) are respectively the triiodinated and tetraiodinated derivates of the thyronine.

While the endocrine determinism of metamorphosis has long been focalized on the thyroid gland activity, it has now been established that it is in fact triggered by the combined effects of a number of hormones [22, 24, 25, 34, 41, 52, 61, 88, 103, 104, 106]. The present article analyzes their combined action on metamorphosis.

I MAIN TYPES OF RESPONSES TO THYROID HORMONES

The vital role of the thyroid hormones in the onset of metamorphosis has prompted many authors to trigger this phenomenon earlier by an artificial and brutal increase in the plasmatic level of T_3 or T_4. Experimental metamorphosis is one of the best methods of analyzing the effects of the thyroid hormones when they enter the cells. The larvae used in these experiments are premetamor-

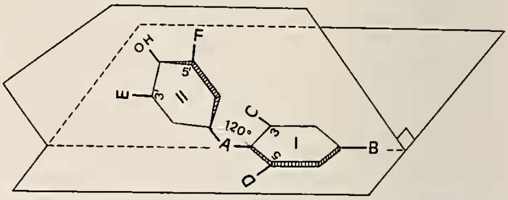


Fig. 2. Structural requirements for thyroid hormone activity on the tail of the anuran tadpole. The hormonally active molecule is composed of two mutually perpendicular aromatic rings (I and II) positioned at an angle of about 120° by a bridging atom, O, S or C (A). An amino acid (alanin, ...) or anionic (acetic acid, ...) side chain of two or three carbons (B) is attached to the aromatic ring I *para* to the bridging atom. A phenolic hydroxyl (-OH) or functionally similar group also *para* to the bridging atom is attached to the aromatic ring II. Both the 3.5 substituents on ring I must be occupied by relatively voluminous groups (C and D) but with diameters no larger than 2.9 nm (methyl radical, halogen, ...). The activity of the basic structure is greatly enhanced by a voluminous substituent (halogen or alkyl radical) in the 3' position (E) on ring II, but reduced by the simultaneous occupancy of the 5' position (F). These results emphasize the thyroid hormone activity is induced by the steric characters of the molecule rather than the specific presence of iodine. The integration of the halogen in the thyroid hormones would be purely accidental and point to an evolution favourable to hormonal metabolism. From Jorgensen [58].

Fig. 4. *In vitro* effects of thyroxine on the tail of an *Alytes obstetricans* premetamorphic tadpole. Fragments were placed in a medium with T_4 at 5.10^{-7} M/ml for 15 days. A phagocytic cell (PH) containing the residual bodies (RB) of heterophagic vacuoles is located between the epidermal cells (EP). DE, dermis. From Pouyet [84].

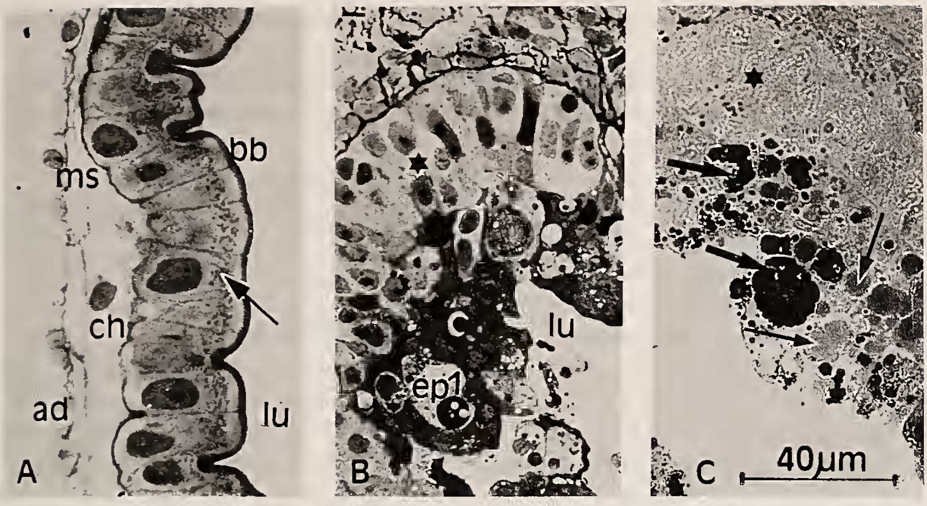
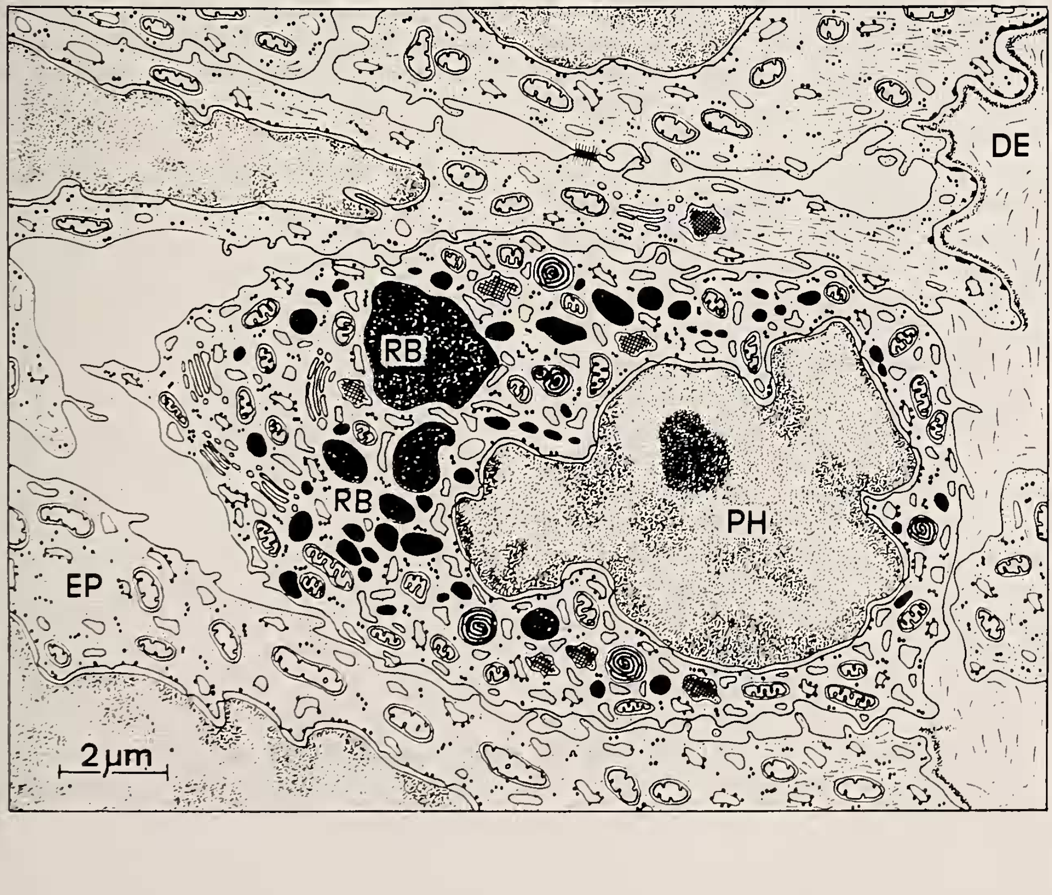


FIG. 3. *Discoglossus pictus* premetamorphic tadpoles treated with T_4: intestine changes induced by the hormone. The animals were placed in an aqueous T_4 solution at 10^{-7} M/ml. A. Intestine section of a control tadpole untreated with T_4. B and C. Intestine section of a sixth-day treated tadpole. B. On the internal border of an islet of embryonic cells (asterisk), the highly chromophilic, lysed primary epithelium (ep1) is being evacuated into the intestinal lumen (lu). C. The primary epithelium is altered by the activity of lysosomal enzymes (acid phosphatase, cathepsins, ...). Acid phosphatase activity is visualized by its black reaction product (thick arrows). ad, adventice; bb, brush border; ch, chorion; ms, muscular sheath. From Hourdry [50].



phic. Some are immerged in a T_3 or T_4 solution with concentrations of 10^{-9} to 10^{-6} M. Others receive intraperitoneal hormone injections with doses of 0.1 to 1 μg/g of body weight. The *in vitro* treatment of tissue explants with T_3 or T_4 often confirms the results obtained *in vivo*.

Treating larvae with thyroid hormones triggers a series of cellular events already encountered during natural metamorphosis (Fig. 2). Certain larval tissues and organs degenerate (tail in anuran tadpoles, gills, digestive tract ...) (Figs. 3 and 4). Cell proliferation centers emerge in the growing limbs, the cerebellum [42] or the digestive tract [53]. The various differentiation stages lead to mature cells and tissues (dendritic arborization of the Purkinje cells in the cerebellum, absorbant brush border in the intestinal epitheliocytes, ...)

Differentiation can be revealed by the accelerated synthesis of molecular species: hydrolytic enzymes in the caudal fibroblasts which become phagocytes, new hemoglobins in the neoformed red blood cells [33], pepsinogen in the chief cells of the stomach, urea-cycle enzymes [5, 111] and serum albumin in the hepatocytes. Finally, cell migrations are observed after the proliferation period, for example, that of the granule cells in the cerebellum.

The larvae treated with thyroid hormones show functional modifications similar to those observed in natural metamorphosis. The animals acquire a saltatory locomotion, a binocular vision and a new hydromineral equilibrium, and become carnivorous. These changes are also associated with a migration of the animals towards the terrestrial habitat [26].

All these experimental changes may be provoked during precocious larval stages, i.e. a long time before the natural metamorphosis. Nevertheless, at first the ability of the tissues to respond to thyroid hormones (competence) is not complete enough to allow integral metamorphosis in the presence of excess hormones. In the beginning, this competence is biochemical and concerns RNA synthesis [2, 20, 21, 76, 85, 102, 104] and that of specialized proteins, before becoming anatomical and morphological. In order to be complete, competence requires tissue aging compatible with a metabolic preparation. When the larvae treated are too young, still partial tissue competence can hinder a complete metamorphic response and lead to an unbalanced development (development of the limbs while the tail is still present ...).

II VARIATIONS AND CONTROL OF THE THYROID HORMONES LEVEL DURING METAMORPHOSIS

A. Circulating hormones

Variations in their level can be observed by radioimmunoassay. The plasma levels of T_3 and T_4 in anurans (*Xenopus, Rana, Bufo ...*) significantly and temporarily increase during climax [69, 74, 75, 87, 98]. The peak values obtained in *Rana catesbeiana* are 70 to 150 ng/100 ml for T_3 and 0.5 to 1 μg/100 ml for T_4, which is the major circulating hormone (Fig. 5). Besides the species, other factors (temperature, light, photoperiod ...) can influence these results. The plasma concentrations of T_3 and T_4 in urodeles (*Ambystoma, Notophthalmus, Eurycea, Hynobius ...*) can also significantly increase during metamorphosis [1, 27, 54, 68, 97].

The main elements of the mechanism controlling

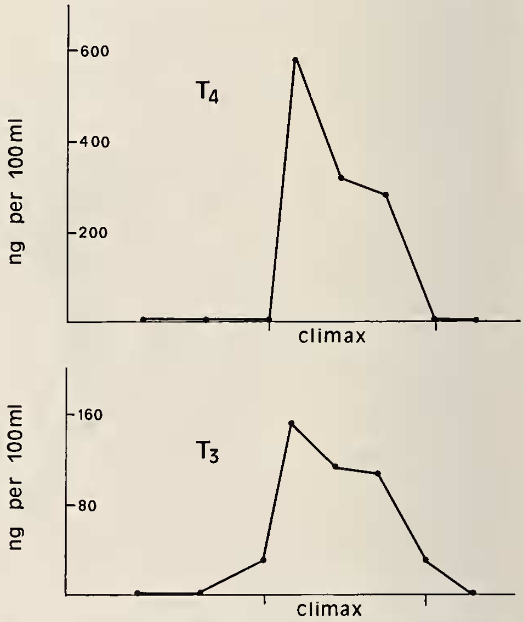


Fig. 5. Plasma T_4 and T_3 levels during development of *Rana catesbeiana* tadpole. The values increase significantly at climax. From Miyauchi *et al.* [74].

the level of circulating thyroid hormones are now well known. Weak thyroid activity during premetamorphosis is due to reduced TSH (thyroid-stimulating hormone or thyrotropic hormone or thyrotropin) secretion by the adenohypophysis. Any unexpected increase in the plasma concentration of thyroid hormones can lead to an interruption of the TSH secretion, due to the negative feedback, and thyroid gland inactivation (Fig. 6).

Just before metamorphosis, the hypothalamus becomes competent towards thyroid hormones and matures considerably (Fig. 7). Neurosecretory centers develop (preoptic nuclei.). A dense capillary network forms at the base of the hypothalamus (median eminence) and connects with the hypothalamo-hypophyseal portal vein. A TRF[1] (thyrotropin releasing factor) secreted by the hypothalamic centers can then stimulate the release of TSH by the adenohypophysis [6, 9, 29]. This leads to an activation of the thyroid gland and an increase in its hormone secretion. The hypothalamus then matures faster (Fig. 6). Through autostimulation of the hypothalamo-hypophyseal-thyroid axis, the concentration of circulating thyroid hormones increases rapidly during metamorphosis.

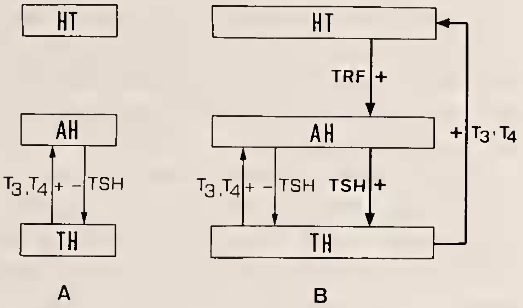


FIG. 6. Activity of the hypothalamo-hypophyseal-thyroid axis during metamorphosis. A. Premetamorphic tadpole. A negative feedback keeps the thyroid activity at a very low level. Any thyroid hormone level increase in the plasma stops TSH (thyroid-stimulating hormone or thyrotropin) secretion by the adenohypophysis. Consequently, the thyroid gland ceases functioning. Moreover, the hypothalamus does not yet control the activity of thyrotropes. B. Metamorphic animal. A self-acceleration of the hypothalamo-hypophyseal-thyroid activity strongly increases the thyroid hormone level in the plasma, through an increasing release of TRF (thyrotropin releasing factor) and TSH. The negative feedback described in A is masked by the stimulatory effects of this positive feedback. AH, adenohypophysis; HT, hypothalamus; TH, thyroid gland. From Etkin [29].

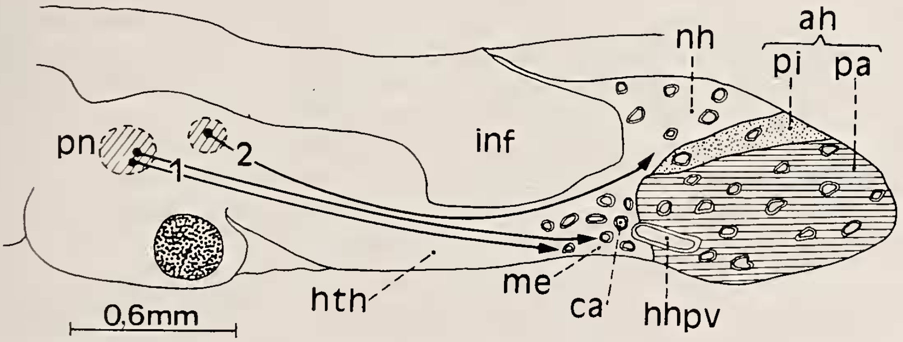


FIG. 7. Hypothalamo-hypophyseal relations in an anuran, *Rana pipiens,* from the climax on. TRF (thyrotropin releasing factor) granules are synthesized in several hypothalamic neurosecretory centers such as preoptic nuclei (pn), and reach the median eminence (me) through axons 1. These granules are then released into blood capillaries (ca) which join a short hypothalamo-hypophyseal portal vein (hhpv). Finally, the TRF granules stimulate the thyrotropes located in the *pars anterior* (or *distalis*) (pa) of the adenohypophysis (ah). Axons 2 carry neurosecretory products towards the neurohypophysis (nh). hth, hypothalamus; inf, infundibulum; pi, *pars intermedia*. From Etkin [28].

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[1] This TRF is different from the TRH (thyrotropin releasing hormone) found in mammals: a tripeptide which up regulates TSH secretion.

When the phenomenon ends, the hypothalamus again becomes insensitive to these hormones and no longer secretes TRF. The thyroid gland then becomes inactive, due to the negative feedback of the adenohypophysis.

An increase in TSH secretion could be triggered not only by TRF but also by hypothalamic neuropeptides more directly involved in the up regulation of ACTH (adrenocorticotropic hormone or corticotropin) or GH (growth hormone) secretion, such as CRF (corticotropin releasing factor) or GHRF (growth hormone releasing factor) [70]. Their chemical origin is shadowy since up to now these peptides have not been isolated in amphibian larvae.

B. Cellular level

At the target cell level, the thyroid hormone concentration is modified by the activity of T_4-5′-monodeiodinase and T_3-5-monodeiodinase, which convert T_4 into T_3 and T_3 into 3,3′-diiodothyronine, respectively [36]. Up to the climax, the activity of T_3-5-monodeiodinase maintains a very low T_3 level in the cells, since the hormone is almost entirely transformed into 3,3′-diiodothyronine. At climax, T_3-5-monodeiodinase activity significantly decreases in certain target cells such as the hepatocytes and red blood cells. Therefore, T_3 cannot be deiodinated. At the same time, T_4-5′-monodeiodinase activity remains weak in these two cell types, thus limiting the production of T_3 from T_4. On the other hand, this activity significantly increases in the skin, intestine and tail, thus leading to a rapid increase in the T_3 level after T_4 deiodination.

Therefore at metamorphosis, the modified activities of T_4-5′-monodeiodinase and T_3-5-monodeiodinase favor the regulation of the thyroid hormones concentration at the cellular level. This concentration was initially determined by these hormones level in the plasma. Thus, the cells can avoid being overexposed to hormones. T_3-5-monodeiodinase for example can inhibit the action of abnormally high T_3 concentrations.

III MECHANISMS OF THYROID HORMONE ACTIVITY IN AMPHIBIAN LARVAE

The different responses to thyroid hormones are prepared by a series of metabolic reactions triggered by the hormones passage into the cells [35, 39]. The delayed response can be detected in treated tissues after about ten days. Furthermore, the response is specific and marks the accomplishment of a preestablished destiny.

A. Thyroid hormone receptors

The competent cells can respond only if the free thyroid hormones are bound to the nuclear receptors, after a thermosensitive transport [46, 101]. The proto-oncogene c-erbA could be the ancestral gene from which the genes coding for these hormone receptors, and more generally for other development signals, come (steroid hormones, retinoic acid, vitamin D3, ...). [31, 44, 90, 93, 107].

Two types of receptors (TRα and TRβ), whose amino acid sequences are similar to those described in chicken or in man, have been deciphered [3, 12]. Small quantities of receptors are found in very young larval tissues. This could explain the early competence of these tissues to hormones. These receptors would then be coded by maternal messengers found in the oocytes. These receptors exist in most of the tadpole cell types (red blood cells, hepatocytes, tail cells or limb buds cells, ...). They can bind one or the other thyroid hormone, but they always have a greater affinity for T_3 than for T_4. The affinity ratio is 2 for hepatocytes and over 15 for red blood cells [36]. This leads to the preferential occupation of the receptors by T_3 at climax. Thus, T_3 is the true metamorphic hormone [36, 37, 69], whether it is secreted directly by the thyroid gland or obtained in the target cells from T_4 after enzymatic deiodination.

In the cells that will undergo significant changes at climax (tail cells that degenerate, totally replaced red blood cells, ...), the number of receptors increases [116], concomitantly with the concentration of circulating hormones. As a result, the competence of the target cells increases. This number is multiplied by 5 in the larval red blood cells, reaching 2500 per nucleus [36], but falls to under 100 in the neoformed red blood cells. However, if the modifications are not as important, as in the hepatocytes undergoing a simple biochemical differentiation, the number of receptors remains about the same. All these results have been confirmed during thyroid hormone treatments.

Gene receptors: relationships between their structure and the coded domains

The TRα and TRβ receptors are each coded by a pair of genes, as shown in *Xenopus laevis* and *Rana catesbeiana* [3, 60, 91, 114, 115]. The structure of the TRβA and TRβB genes, coding the TRβ receptors, has recently been analyzed in *Xenopus laevis* [59, 94, 115] (Fig. 8). These genes are 70kbp long. The TRβA gene has 14 exons and the TRβB has 12 exons. Each one has 2 promoters, located upstream from exons a and b respectively.

Only one initiates TRβ-mRNA transcription in the presence of thyroid hormones, after RNA polymerase binding. The a-i exons code RNA sequences which are poorly translated and give very short domains.

The TRβ receptors are more specifically translated from exons 1–6 (Fig. 8). The DNA binding domain (DNA-B) is essentially made up of two folds in the amino acid chain, whose stability is guaranteed by the binding of a zinc atom with 4 cystein molecules. This particular shape accounts for the name "zinc fingers" given to these structures [66]. The first "zinc finger" is coded by exon 1: it is bound to DNA by a specific sequence of bases it recognizes (hormone response element or HRE). The second "zinc finger" is coded by exon 2: it represents a stabilizing element and keeps the recognition structure in place. The thyroid hormone binding domain (TH-B) is coded by exons 4–6. Exon 3 furnishes a flexible hinge, connecting DNA-B and TH-B. The structure of the thyroid hormone receptor is schematized in Fig. 8.

Receptor messengers

The information concerning the genes coding the

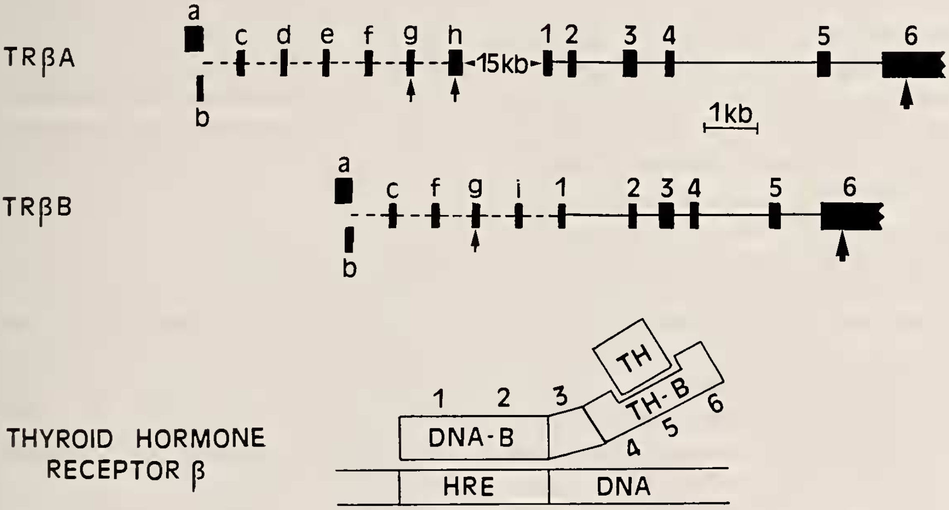

FIG. 8. Organization of the two *Xenopus laevis* TRβ genes: TRβA and TRβB. The exons a-i encode a mostly untranslated region, and exons 1–6 encode the thyroid hormone receptor β (=TRβ). Exon 6 of both genes is the largest exon. The relative locations of exons a and b are unknown and are, therefore, shown in the same position. Each of the TRβ genes has two promoters, one located upstream of exon a and the other upstream of exon b. The translation initiation and termination sites are indicated by thin and thick arrows, respectively. At the bottom of the figure, the thyroid hormone receptor β consists of two distinct functional domains including the DNA- and hormone-binding domains (DNA-B and TH-B, respectively). It binds to a specific DNA element called the hormone response element (HRE). The two "zinc fingers" of DNA-B are encoded by exons 1 and 2, and TH-B is split into exons 4–6. DNA-B and TH-B are linked by a domain encoded by exon 3. TH, thyroid hormone. From Shi *et al.* [94] and Yaoita *et al.* [115].

receptors has been completed by a precise analysis of the mechanisms of messenger transcription [3, 4, 60, 114]. The level of TRα-mRNAs triples during spontaneous metamorphosis. The level of TRβ-mRNAs is 10 to 30 times lower during premetamorphosis. It increases very rapidly at climax, when it can multiply by 10 or more, concomitantly with the increase in the circulating thyroid hormone concentration, as metamorphosis progresses. The level of TRβ-mRNAs decreases after. Higher TRβ-mRNAs levels are also obtained after thyroid hormone treatments. Finally, the latter up-regulate the transcription of TRβ-mRNAs more than that of TRα-mRNAs, prior to the true auto-induction of the receptors by their own hormones. Furthermore, TRβ-mRNAs transcription represents one of the earliest responses to T_3 or T_4. *In situ* hybridization experiments confirm the variable location of TR-mRNA in one tissue or another [60].

The rate or TRβ-mRNAs transcription in the presence of thyroid hormones is not the same if the promoter is carried by exon a or b. After a 2-day treatment, transcription is intiated on the promoter upstream from exon b and becomes very rapid, its rate increasing at least tenfold [59]. After 7 days of treatment, the transcription triggered by the promoter on exon a accelerates. The TRβA and TRβB gene promoters could therefore initiate cascades of early or late gene expressions whether they belong to exon b or a.

Once the thyroid hormones are positioned on their receptors, they influence the regulatory gene network, including promoters, which in turn controls the structural genes activity according to mechanisms which are now well-known [2, 20, 21, 60, 71, 77, 102, 104, 115]. The intimate mechanisms of gene activation or repression however remain obscure.

B. Thyroid hormones and cell division

The cell multiplication which takes place in the various larval tissues in the presence of thyroid hormones implies an up-regulation of nuclear DNA synthesis. This up-regulation can be visualized by radioautography which measures the frequency of radioactive, silver-labelled nuclei (labelling index), after radioprecursor (tritiated thymidine) incorporation by DNA. The labelling index increases in the lengthening anlages, the lungs and the new-digestive epithelium, among others [52].

C. Inducing cell differentiation and degeneration

These events are triggered by a modification in gene activity after the thyroid hormones bind to their receptors. Certain genes are activated and allow the accelerated transcription of the RNA messengers in the target cells. Other genes that were functional up to then are repressed. According to Gruenstein [47], the thyroid hormones control gene activity through their action on the protein fraction of chromatin and particularly on the histones, whose role had long remained unclear. The hormones would unbind the H4 histone linked to the TATA box[1] of certain gene promoters, via activating proteins. Following a modification in the configuration of this histone, the nucleosomes containing it, as well as histones H2A, H2B and H3, could temporarily cease being compact masses. The DNA surrounding these nucleosomes could partially uncoil, and the genes could then be transcribed thanks to the bases greater accessibility and the two RNA-polymerases activity [45]. These genes would therefore stops being repressed. On the contrary, their repression could be triggered by the reconstitution of compact nucleosomes on which the genic material coils.

Protein synthesis control

In larvae undergoing metamorphic changes prior to their departure from water, thyroid hormones stimulate specialized protein synthesis in the degenerating or functionally differentiating cells, after a latency period due to the preliminary transcription of a larger number of messengers [4, 15, 52, 72, 78]. (Fig. 9).

The variations in hepatic proteosynthesis in anuran tadpoles treated with T_3 or T_4 have been investigated in numerous cytological and biochemical studies. Treating premetamorphic animals with one or the other hormone up-regulates RNA transcription and membrane phospholipid synthe-

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[1] The TATA box includes a nucleotide sequence derived from the TATAAATA sequence.

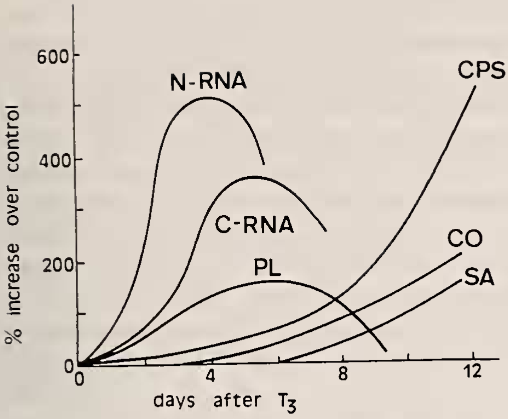

Fig. 9. Representation of sequential stimulation of RNA and phospholipid synthesis rates in relation to the increase in enzymes and proteins synthesized during the precocious induction of metamorphosis in *Rana catesbeiana* tadpoles with T_3. Nuclear RNA (N-RNA) synthesis increases up to the fourth day. Then, RNAs are stored in cytoplasmic ribosomes and polysomes (C-RNA). The rate of microsomal phospholipid synthesis increases up to the 6th day (PL). After the 7th day, specialized proteins are synthesized in the hepatocytes. They will become localized in the mitochondria (carbamyl phosphate synthetase-I: CPS; cytochrome oxidase: CO), or will be secreted into the plasma (serum albumin: SA). The values are expressed as percentage increases over those in the non-induced control tadpoles. From Tata [100].

sis. At the same time, the hepatocytes show signs of cytological differentiation, with the arrangement of the ribosomes into numerous polysomes bound to a more abundant endoplasmic reticulum. Serum albumin, urea-cycle enzymes (carbamyl phosphate synthetase-I, ...) or energy catabolism enzymes (cytochrome oxidase, ...) are among the more actively coded proteins. These synthesis have a deep-seated influence on the tadpoles physiology. For example, urea-cycle activation enhances urea production by the liver and induces ureotelia. Furhermore, the afflux of serum albumin in the blood increases its osmotic pressure, which leads to a more efficient water conservation during the transition to a terrestrial environment. The intimate proteosynthetic mechanisms have been more specifically studied for carbamyl phosphate synthetase-I which integrates ammonia into the urea-cycle. The synthesis of this enzyme is up-regulated in under 24 hours in the hepatocytes of premetamorphic *Rana catesbeiana* tadpoles treated with T_3 or T_4. Two sub-units are rapidly coded at the polysome level, then transferred to the mitochondria where they assemble in the matrix, thus generating the active enzyme [76]. This enzyme has a relatively long life-span and continues to accumulate in the liver, contrary to the messengers which are rapidly eliminated [40]. Under *in vitro* conditions, the same hormones up-regulate the albumin messengers transcription, followed by their translation which has been quantified using antibodies [92].

The skin of anuran tadpoles is also a target organ where the thyroid hormones can up-regulate specific proteosynthesis. Indeed, under *in vitro* conditions [72] or during spontaneous metamorphosis, these hormones activate the genes of the three new keratins which replace those of the larvae, while the epidermis thickens by cell multiplication.

The Hb.l[1] polypeptide chains in various anuran tadpoles (*Rana catesbeiana, ...*) stop being produced after a thyroid hormone treatment due to the down-regulation of the corresponding genes. On the other hand, the genes coding the Hb.ad[1] polypeptide chains are up-regulated [79, 110]. Such modifications are associated with the appearance of new red blood cells.

The exogenous thyroid hormones increase the production of lysosomal hydrolases (cathepsin D, ...) in the tail fibroblasts of anuran tadpoles, after gene activation [89]. Concomitantly, these cells acquire a greater mobility and contribute to tail regression by phagocyting the tissue remnants.

The lysosomal hydrolases activities have also been visualized in various degenerating caudal cells, after a T_3 or T_4 treatment in anuran tadpoles. Apart from hydrolase synthesis, the hormones can also induce these cells to produce activating proteins, which in turn reactivate preexisting but inactive lysosomal prohydrolases [8].

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[1] The Hb.l and Hb.ad are the hemoglobins of the larva and the adult, respectively.

IV OTHER METAMORPHIC UP-REGULATING HORMONES: CORTICAL STEROIDS

The main cortical steroids produced by amphibian interrenals are corticosterone and aldosterone. These stroids can accelerate spontaneous or thyroid hormone-tirggered metamorphosis in amphibians. A simultaneous T_3 and cortical steroid treatment in larvae leads to faster transformations than a simple T_3 treatment. The tail in anuran tadpoles and caudal fin in urodelean larvae degenerate twice as fast [16, 38, 64]. Certain metamorphic biochemical events such as carbamyl phosphate synthetase-I synthesis by the liver are also up-regulated [38]. In fact, aldosterone seems to be more efficient than corticosterone. The latency period prior to the appearance of a metamorphic response is itself shortened. On the other hand, metamorphosis is checked, if the anuran tadpoles are simultaneously treated with a thyroid hormone and amphenone B or any other corticosteroid synthesis inhibitor [62]. A significant delay in caudal resorption can then be observed. This

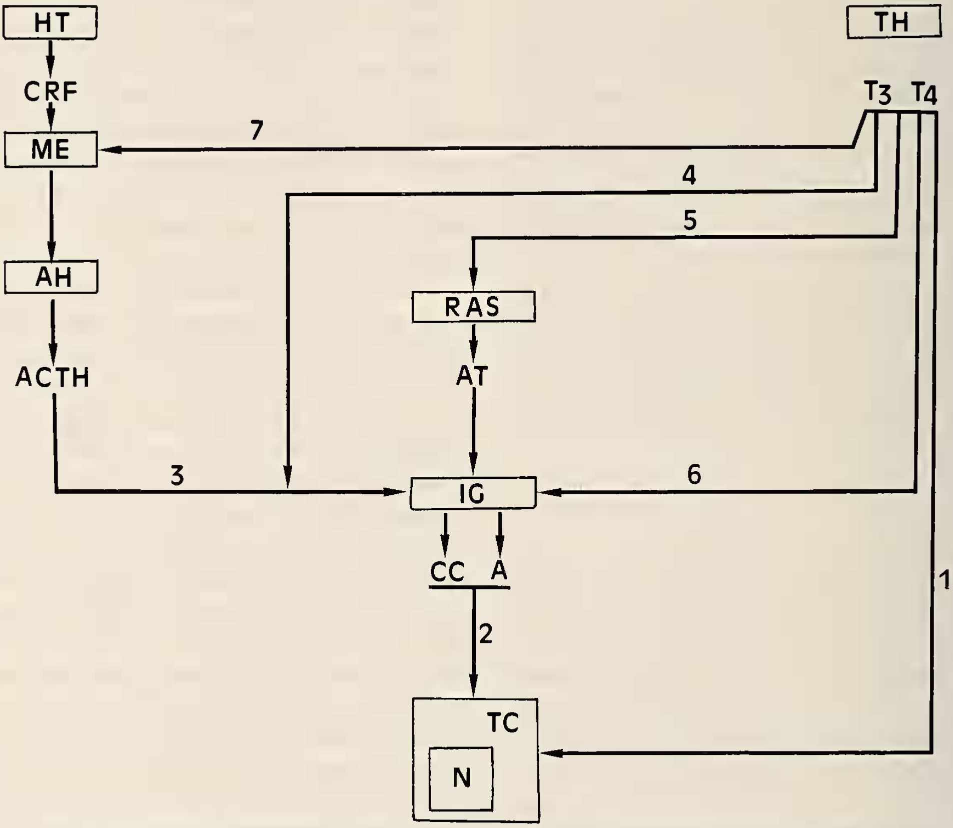

Fig. 10. Interactions between thyroid hormones (T_3, T_4) and corticoids (corticosterone: CC, aldosterone: A) from the interrenal glands (IG) in the stimulation of amphibian metamorphosis. Thyroid hormones bind to nuclei (N) of target cells (TC) to induce the enzymes that participate in the metamorphic events (1). The binding is enhanced by interrenal steroids (2). The secretion of these steroids is stimulated by adrenocorticotropic hormone (ACTH) (3) whose action is enhanced by thyroid hormones (4). Thyroid hormones also stimulate the secretion of aldosterone and corticosterone through the activation of the renin-angiotensin system (RAS) (5) or directly, possibly by the direct activation of interrenal cells (6). Thyroid hormones induce the differentiation of the median eminence (ME) (7), thus facilitating the flow of a corticotropic releasing factor (CRF) from the hypothalamus (HT) to stimulate the corticotropes of the adenophypophysis (AH) in the release of ACTH. AT, angiotensin; TH, thyroid gland. From Dent [22].

confirms the part played by endogenous cortical steroids as well as the thyroid hormones in up-regulating metamorphosis.

Hydrocortisone has little potentiating effect on thyroid hormones in toad and bullfrog larvae, although exogenous hydrocortisone has been reported to act synergistically with thyroid hormones to increase keratic gene expression in *Xenopus* epidermis [95].

Endogenous cortical steroids are more abundantly secreted by the interrenal glands during spontaneous metamorphosis. Various radioimmunoassays have demonstrated this in anurans and urodeles for corticosterone [16, 55, 57] and for aldosterone [57, 64]. The up-regulated interrenal metabolism in metamorphic amphibians is further emphasized by a stronger hormonogenic enzyme activity, as for 3β-hydroxysteroid dehydrogenase [16].

Thyroid hormones can increase the level of circulating corticosteroids by acting at the hypothalamo-hypophyseal level. Indeed, CRF transit from the hypothalamus to the adenohypophysis improves as does that of TRF thanks to a larger capillarisation of the median eminence. The corticotropic cells then secrete more ACTH [22, 64], which results in an up-regulation of the interrenals. Other types of thyroid hormone activity, such as a direct effect on the synthesis of the enzymes needed for interrenal metabolism and an activation of the renin-angiotensin system improving aldosterone release, have been postulated [64] (Fig. 10).

Corticosteroids could favor T_4 conversion to T_3 (active form) by up-regulating the activity of T_4-5′-monodeiodinase, in the skin for example. They could also limit T_3 deiodination into inactive diiodothyronine by slowing down T_3-5-monodeiodinase activity, as shown in the liver and intestine [38]. In both cases, the concentration of T_3 increases. Corticosteroids potentiate also T_3 action on the tail *in vitro* [63]. This indicates that another mechanism of corticosteroid-thyroid hormone action exists. The influence of corticosteroids on the number of receptors per nucleus is still being discussed. According to Galton [38], this number would remain the same in the hepatocytes, caudal cells and red blood cells after treatment. On the other hand, corticosteroids could enhance the metamorphic action of thyroid hormones by increasing their binding capacity to the nuclear receptors in the target cells [22, 96].

Finally, while metamorphosis is triggered by thyroid hormones, cortical steroids have an authentic up-regulating effect on these hormones.

V THE METAMORPHOSIS INHIBITING HORMONES

A. Prolactin

Prolactin is secreted by the adenohypophysis. Exogenous prolactin inhibits certain natural or induced [10, 14, 30, 51, 80] or *in vitro* [99] metamorphic events in anuran larvae. It prevents tail resorption and hindlimb development. Concomitantly, the transition to a terrestrial environment is eliminated or delayed. Injecting prolactin also inhibits urodelean metamorphosis and delays the appearance of juvenile characteristics [19, 43]. The gills remain practically intact, while emergence from water becomes more difficult. A treatment with homologous prolactin produces analogous results [65].

Therefore, a prolactin treatment in larvae triggers effects opposite to those of thyroid hormones. This reflects a double antagonism. Prolactin works against certain effects of T_4 and T_3 on the tissues [4, 11, 23, 56, 83, 105] and reduces these hormones secretion by inactivating the thyroid gland [14, 43, 86]. At tissue level, the up-regulation of TRα- and TRβ-mRNAs transcription by T_3 could be inhibited by prolactin in both the premetamorphic tadpole and tail organ cultures of *Xenopus laevis* [4]. Under such conditions, the small number of coded receptors would not allow gene activation by T_3 and continued proteosynthesis. Prolactin could also favor the production of a hepatic factor (synlactin) up-regulating tail growth.

Prolactin cells have been visualized by immunocytochemistry or immunofluorescence in various anurans (*Rana catesbeiana, Bufo japonicus* ...) and urodeles (*Pleurodeles waltl* ...). The number of cells as well as the quantity of prolactin contained in the adenohypophysis peak at climax [18, 81, 113]. The level of plasma prolactin has been determined by radioimmunoassay in several anurans (*Rana catesbeiana, Bufo japonicus* ...). This level peaks at climax and decreases after [18, 81, 112, 113] (Fig. 11). Nevertheless, these physiological values would be too weak to inhibit metamorphic events, contrary to pharmacological values arising from a prolactin treatment.

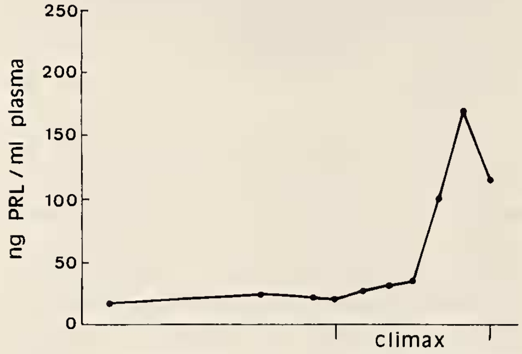

FIG. 11. Plasma prolactin (PRL) level during development of *Rana catesbeiana* tadpole. The values increase significantly towards the end of climax. From Yamamoto and Kikuyama [112].

B. Growth hormone

Treatment with the growth hormone, also secreted by the adenohypophysis, can inhibit certain metamorphic events such as tail resorption. Nevertheless, its effects seem to be weaker than those of prolactin [73]. The plasma growth hormone concentration peaks towards the end of metamorphosis and decreases in juveniles, then increases again in adults [18]. According to Kobayashi and Kikuyama [67], who extensively measured growth hormone levels in tadpoles throughout metamorphosis and juveniles and various sizes of male and female adults in the same species (*Rana catesbeiana*), the pattern of hormone concentrations would be slightly different. Growth hormone levels rose as metamorphosis progresses and showed the higher values in juveniles, which show a rapid growth, and declined as they gain weight.

VI THE HYDROMINERAL EQUILIBRIUM REGULATING HORMONES

Emergence from water in metamorphosed amphibians is associated with a modification in the hydromineral equilibrium, which optimizes water conservation by the organism [52]

A. The neurohypophyseal hormones

The neurohypophyseal hormones more specifically contribute to controlling the hydromineral equilibrium. In amphibians, the main hormone concerned is arginine vasotocin (AVT), a nanopeptide that includes leucine in position 3 and arginine in 8. An AVT treatment induces antidiuresis in metamorphosed anurans which have become terrestrial (*Rana, Bufo* ...): glomerular filtration slows down and water is reabsorbed in large quantities by the renal tubules and urinary bladder. The hormone also acts at skin level by up-regulating osmotic water absorption. On the other hand, in adult *Xenopus* which can remain in an aquatic environment after metamorphosis, an AVT treatment increases the diuretic effect also observed in fresh water fish. These observations show that from the juvenile phase on, AVT enhances hydric retention in anurans proportionally to their degree of adaptation to a terrestrial environment.

The neurohypophysis in anuran larvae is too small for AVT to be detected. Its volume increases in metamorphosing anurans and its capillary network becomes denser. These two observations suggest an accelerated AVT secretion, which would improve water conservation in anurans migrating to a terrestrial environment.

B. Aldosterone

The epidermis of metamorphosed amphibians can pick up sodium ions in fresh water by active trasnsport against a concentration gradient. This cannot be done by the larval epidermis. This ionic flux is associated with water penetration by osmosis, through the skin, each time the now terrestrial animals return to an aquatic environment. The epidermal cells in metamorphosed amphibians become permeable to sodium ions, when sodic channels appear in the apical plasma membrane under the influence of thyroid hormones [99]. Active Na^+ transport occurs only through the baso-lateral plasma membrane. This type of transport, which modulates ion recuperation (and therefore that of water), is more specifically controlled by aldosterone [99].

The epithelium of the urinary bladder, another water- and ion-recuperating organ, could also have the same type of hormonal regulation.

C. Prolactin

A prolactin treatment improves water, sodium and chloride ion recovery by the kidney and urinary bladder, in metamorphosed amphibians that have become terrestrial. The hormone helps the animals adapt to a terrestrial environment by allowing them to save water more efficiently. Prolactin control of the hydromineral equilibrium through the kidney and urinary bladder is made possible by the fact that these organs acquire numerous prolactin receptors during metamorphosis [13, 49, 82, 108, 109].

In actual fact, the pharmacological doses due to a prolactin treatment would produce opposite effects with regard to the departure from water. Hormone injections could prevent this ecological transition by inhibiting certain metamorphic events. On the other hand, they could adapt better metamorphosed animals to a terrestrial habitat by enhancing hydric retention.

VII THE HORMONES CONTROLLING ENERGY METABOLISM

During metamorphosis and the departure from water, the intense morphogenetic processes require an up-regulation of energy metabolism and a mobilization of the lipid and glucid reserves. This is controlled by the newly installed endocrine system. Cortical steroids (aldosterone, corticosterone) from the interrenal cells and probably epinephrine and norepinephrine from the adrenal cells are secreted into the plasma [55]. These hormones improve the reserves mobilization[1] and the hyperglycemic response. This in turn leads to the maturation of the islets of Langerhans in the pancreas and higher insulin secretion [7, 17, 32]. Endocrine control in amphibians however does not imply stable glycemia as in mammals. This control remains imperfect to the point of furnishing an inadequately adapted response to the animals energy needs. This could be a potential problem in the transition to a terrestrial environment, where outside elements vary more than in water and require a permanent adjustment of the energy needs.

GENERAL CONCLUSIONS

In many amphibians, the metamorphic events associated with the departure from water are controlled by a number of hormones. The most important are the thyroid hormones. After they bind to the nuclear receptors, the thyroid hormones trigger a number of mechanisms by acting at the genome level. These hormones control the synthesis of many specialized proteins during cell differentiation and degeneration. The succession of metabolic stages explains the latency of the responses.

In amphibians with a deficient hypothalamo-hypophyseal-thyroid axis such as axolotl or anuran larvae maintained in cold conditions, natural metamorphic changes are very limited. Such perpetually larval, aquatic animals have been qualified as neotenic *sensu lato*. The persistence of a larval stage in axolotl is nevertheless associated with the ability to reproduce (neoteny *sensu stricto*). Treating axolotl with thyroid hormones leads to metamorphosis and the departure from water. This proves the existence of competent tissues, contrary to what is observed in various compulsory neotenic urodeles (*Necturus, Proteus*).

Thyroid hormone activity is a necessary condition for metamorphosis and departure from water in many amphibians. However, it is not in itself sufficient, since certain metamorphosed species such as *Xenopus* remain in fresh water.

This suggests the contribution of other hormones whose action would differ according to the species. Thus, AVT is antidiuretic in the frog, making water conservation easier in a terrestrial environment, while it has a diuretic effect incompatible with a departure from water in *Xenopus*. A prolactin treatment can inhibit metamorphosis in anuran larvae, thus maintaining them in an aquatic environment. The same treatment in various juvenile terrestrial newts (*Notophthalmus viridescens*) anticipates "puberty metamorphosis" ("second metamorphosis") prior to a premature return to water. Prolactin activity can thus be favorable to an aquatic environment, at least under experimental conditions.

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[1] Cortical steroids could make metamorphosis easier by allowing a more efficient energetic response. Metamorphosis could be significantly delayed if their synthesis is inhibited in any way [62].

Finally, a complex endocrine determinism presides over the environmental transitions observed in modern amphibian development.

REFERENCES

1 Alberch P, Gale EA, Larsen PR (1986) Gen Comp Endocrinol 61: 153–163

2 Atkinson BG (1971) In "Relationships of Endocrines to Growth and Development" Ed by RP Breitenbach, AD Kenny, University of Missouri, Columbia, pp 48–82

3 Baker BS, Tata JR (1990) EMBO J 9: 879–885

4 Baker BS, Tata JR (1992) Dev Biol 149: 463–467

5 Balinsky JB, Coetzer TL, Mattheyse FJ (1972) Comp Biochem Physiol 43B: 83–95

6 Ball JN (1981) Gen Comp Endocrinol 44: 135–170

7 Bartell MH, Frye BE (1974) J Exp Zool 190: 175–184

8 Beckingham-Smith K, Tata JR (1976) Exp Cell Res 100: 129–146

9 Belensky MA, Chetverukhin VK, Polenov AL (1973) Gen Comp Endocrinol 21: 241–261

10 Bern HA, Nicoll CS, Strohman RC (1967) Proc Soc Exp Biol Med 126: 518–520

11 Blatt LM, Slickers KA, Kim KH (1969) Endocrinology 85: 1213–1215

12 Brooks AR, Sweeney G, Old RW (1989) Nucl Acids Res 17: 9395–9405

13 Brown PS, Brown SC (1987) In "Vertebrate Endocrinology: Fundamentals and Biomedical Implications" Ed by PKT Pang, MP Schreibman, Academic Press, New York, pp 45–84

14 Brown PS, Frye BE (1969) Gen Comp Endocrinol 13: 126–138

15 Buckbinder L, Brown DD (1992) J Biol Chem 267: 25786–25791

16 Carr JA, Norris DO (1988) Gen Comp Endocrinol 71: 63–69

17 Cheng-Kaung HL (1983) Gen Comp Endocrinol 49: 50–56

18 Clemons GK, Nicoll CS (1977) Gen Comp Endocrinol 32: 531–535

19 Cohen DC, Greenberg JA, Licht P, Bern HA, Zipser RD (1972) Gen Comp Endocrinol 18: 384–390

20 Cohen PP (1970) Science 168: 533–543

21 Cohen PP, Brucker RF, Morris SM (1978) In "Hormonal Proteins and Peptides. Thyroid Hormones Vol 6" Ed by CH Li, Academic Press, New York, pp 273–381

22 Dent JN (1988) Am Zool 28: 297–308

23 Derby A, Etkin W (1968) J Exp Zool 169: 1–8

24 Dodd MHI, Dodd JM (1976) In "Physiology of the Amphibia Vol 3" Ed B Lofts, Academic Press, New York, pp 467–599

25 Duellman WE, Trueb L (1986) Biology of Amphibians, McGraw-Hill Book Company, Montreal

26 Duvall D, Norris DO (1980) Animal Behav 28: 116–123

27 Eagleson GW, McKeown BA (1978) Can J Zool 56: 1377–1381

28 Etkin W (1965) J Morphol 116: 371–378

29 Etkin W (1968) In "Metamorphosis: a Problem in Developmental Biology" Ed by W Etkin, LI Gilbert, Appleton-Century-Crofts, New York, pp 313–348

30 Etkin W, Gona AG (1967) J Exp Zool 165: 249–258

31 Evans RM (1988) Science 240: 889–895

32 Farrar ES, Hulsebius JJ (1988) Gen Comp Endocrinol 69: 65–70

33 Flavin M, Duprat AM, Rosa J (1982) Cell Differ 11: 27–33

34 Frieden E, Just JJ (1970) In "Biochemical Actions of Hormones Vol 1" Ed by G Litwack, Academic Press, New York, pp 1–52

35 Galton VA (1983) In "Molecular Basis of Thyroid Hormone Action" Ed by JH Oppenheimer, HH Samuels, Academic Press, New York, pp 445–483

36 Galton VA (1988) Am Zool 28: 309–318

37 Galton VA (1989) Endocrinology 124: 2427–2433

38 Galton VA (1990) Endocrinology 127: 2997–3008

39 Galton VA (1992) Trends in Endocrinol Metab 3: 96–100

40 Galton VA, Morganelli CM, Schneider MJ, Yee K (1991) Endocrinology 129: 2298–2304

41 Gilbert LI, Frieden E (1981) Metamorphosis: a Problem in Developmental Biology (second edition). Plenum Publishing Corporation, New York.

42 Gona AG (1973) Exp Neurol 38: 494–501

43 Gona AG, Etkin W (1970) Gen Comp Endocrinol 14: 589–591

44 Green S, Chambon P (1986) Nature 324: 615–617

45 Griswold MD, Cohen PP (1972) J Biol Chem 247: 353–359

46 Griswold MD, Fischer MS, Cohen PP (1972) Proc Natl Acad Sci USA 69: 1486–1489

47 Grunstein M (1992) Pour la Science 182: 48–56

48 Gudernatsch JF (1912) Arch (W Roux) Entwickl Mech Organ 35: 457–483

49 Hirano T, Ogasawara T, Bolton JP, Collie NL, Hasegawa S, Iwata M (1987) In "Comparative Physiology of Environmental Adaptation" Ed by R Kirsch, B Lahlou, Karger, Basel, pp 112–124

50 Hourdry J (1971) J Microsc 12: 205–224

51 Hourdry J (1978) Année Biol, 17: 557-569

52 Hourdry J, Beaumont A (1985) Les Métamorphoses des Amphibiens. Masson/Singer-Polignac, Paris

53 Hourdry J, Dauça M (1977) Int Rev Cytol Suppl 5: 337–385

54 Jacobs GFM, Michielsen RPA, Kühn ER (1988) Gen Comp Endocrinol 70: 145–151

55 Jaffe RC (1981) Gen Comp Endocrinol 44: 314–318

56 Jaffe RC, Geschwind II (1974) Gen Comp Endocrinol 22: 289–295

57 Jolivet-Jaudet G, Leloup-Hatey J (1984) Gen Comp Endocrinol 56: 59–65

58 Jorgensen EC (1978) In "Hormonal Proteins and Peptides. Thyroid Hormones Vol 6" Ed by CH Li, Academic Press, New York, pp 107–204

59 Kanamori A, Brown DD (1992) J Biol Chem 267: 739–745

60 Kawahara A, Baker BS, Tata JR (1991) Development 112: 933–943

61 Kikuyama S, Kawamura K, Tanaka S, Yamamoto K (1993) Int Rev Cytol 145: 105–148

62 Kikuyama S, Niki K, Mayumi M, Kawamura K (1983) Endocrinol Jpn 29: 659–662

63 Kikuyama S, Niki K, Mayumi M, Shibayama R, Nishikawa, M, Shintake N (1983) Gen Comp Endocrinol 52: 395–399

64 Kikuyama S, Suzuki MR, Iwamuro S (1986) Gen Comp Endocrinol 63: 186–190

65 Kikuyama S, Yamamoto K, Mayumi M (1980) Gen Comp Endocrinol 41: 212–216

66 Klug A, Rhodes D (1987) Trends Biochem Sci 12: 464–469

67 Kobayashi T, Kikuyama S (1991) Gen Comp Endocrinol 82: 14–22

68 Larras-Regard E, Taurog A, Dorris ML (1981) Gen Comp Endocrinol 43: 443–450

69 Leloup J, Buscaglia M (1977) CR Acad Sci Fr 284: 2261–2263

70 Licht P (1990) J Exp Zool suppl 4: 159–160

71 Lyman DF, White BA (1987) J Biol Chem 262: 5233–5237

72 Mathisen PM, Miller L (1989) Mol Cell Biol 9: 1823–1831

73 Medda AK, Frieden E (1970) Endocrinology 87: 356–365

74 Miyauchi H, La Rochelle FT, Suzuki M, Freeman M, Frieden E (1977) Gen Comp Endocrinol 33: 254–266

75 Mondou PM, Kaltenbach JC (1979) Gen Comp Endocrinol 39: 343–349

76 Mori M, Morris SM, Cohen PP (1979) Proc Natl Acad Sci USA 76: 3179–3183

77 Morris JM (1987) Arch Biochem Biophys 259: 144–148

78 Moskaitis JE, Sargent TD, Smith LH Jr, Pastori RL, Schoenberg DR (1989) Mol Endocrinol 3: 464–473

79 Moss B, Ingram VM (1968) J Mol Biol 32: 481–504

80 Nicoll CS (1974) In "Handbook of Physiology, Section 7: Endocrinology Vol 4" Ed by E Knobil, WH Sawyer, Williams and Wilkins, Baltimore, pp 253–292

81 Niinuma K, Yamamoto K, Kikuyama S (1991) Zool Sci 8: 97–101

82 Olivereau M, Olivereau JM, Kikuyama S, Yamamoto K (1990) In "Fortschritte der Zoologie: Biology and Physiology of Amphibians Vol 38" Ed by W Hanke, Gustav Fischer Verlag, Stuttgart, pp 371–383

83 Platt JE, Christopher MA (1977) Gen Comp Endocrinol 31: 243–248

84 Pouyet JC (1976) CR Soc Biol Fr 170: 942–945

85 Reeves R (1977) Dev Biol 60: 163–179

86 Regard E, Hourdry J (1975) J Microsc 22: 39–54

87 Regard E, Taurog A, Nakashima T (1978) Endocrinology 102: 674–684

88 Rosenkilde P (1979) In "Hormones and Evolution Vol 1" Ed by EJW Barrington, Academic Press, New York, pp 437–491

89 Ryffel G, Weber R (1973) Exp Cell Res 77: 79–88

90 Sap J, Munoz A, Damm K, Goldberg Y, Ghysdael J, Leutz A, Beug H, Vennstrom B (1986) Nature 324: 635–640

91 Schneider MJ, Galton VA (1991) Mol Endocrinol 5: 201–208

92 Schultz JJ, Price MP, Frieden E (1988) J Exp Zool 247: 69–76

93 Shepel LA, Gorski J (1988) Biofactors 1: 71–83

94 Shi YB, Yaoita Y, Brown DD (1992) J Biol Chem 267: 733–738

95 Shimizu-Nishikawa K, Miller L (1992) Differentiation 49: 77–83

96 Suzuki MR, Kikuyama S (1983) Gen Comp Endocrinol 52: 272–278

97 Suzuki S (1987) Zool Sci 4: 849–854

98 Suzuki S, Suzuki M (1981) Gen Comp Endocrinol 45: 74–81

99 Takada M (1990) Gen Comp Endocrinol 77: 442–

447

100 Tata JR (1967) Biochem J 105: 783–801

101 Tata JR (1968) Dev Biol 18: 415–440

102 Tata JR (1971) In "Control Mechanisms of Growth and Differentiation Vol 25" University Press, Cambridge, pp 163–181

103 Tata JR (1973) In "Metamorphosis Vol 46" Ed by JJ Head, OE Lowenstein, Oxford Biology Readers, pp 1–16

104 Tata JR (1974) In "Handbook of Physiology, Section 7: Endocrinology Vol 3" Ed by RO Greep, EB Astwood, Williams and Wilkins, London pp 469–478

105 Tata JR, Kawahara A, Baker BS (1991) Dev Biol 146: 72–80

106 Weber R (1967) In "The Biochemistry of Animal Development Vol 2" Ed by R Weber, Academic Press, New York, pp 227–301

107 Weinberger C, Thompson CC, Ong ES, Lebo R, Gruol, DJ, Evans RM (1986) Nature 324: 641–646

108 White BA (1981) Gen Comp Endocrinol 45: 153–161

109 White BA, Nicoll CS (1979) Science 204: 851–853

110 Widmer HJ, Andres AC, Niessing J, Hosbach HA, Weber R (1981) Dev Biol 88: 325–332

111 Wixom RL, Reddy MK, Cohen PP (1972) J Biol Chem 247: 3684–3692

112 Yamamoto K, Kikuyama S (1982) Endocrinol Jpn 29: 159–167

113 Yamamoto K, Niinuma K, Kikuyama S (1986) Gen Comp Endocrinol 62: 247–253

114 Yaoita Y, Brown DD (1990) Genes Dev 4: 1917–1924

115 Yaoita Y, Shi Y, Brown DD (1990) Proc Natl Acad Sci USA 87: 7090–7094

116 Yoshizato K, Frieden E (1975) Nature 254: 705–707